

BEST AVAILABLE COPY

2

Repeat wt-1 peptide binding Assay.

5x10⁵ T2 cells / well in RPMI + 5% Boiled FCS.

Add peptides to a final conc. of 100 nM.

Leave to binding at 37°C 7h

Wash x1. with PBS + 2% FCS.

Apply mAb A2 mAb HB34. HB117 30' on ice

Wash x3

Apply GAM FITC (250) 30' on ice

Wash x3

Resuspend cells in PBS (400ul).

FACS Analysis

Results, Good.

peptide

Mean: HB34

HB117

— mAb only 5.3

HPV E7/86-93

HBV

WT-1 / 10-

WT-1 / 17-

WT-1 / 126-

WT-1 / 187-

WT-1 / 225-

WT-1 / 235-

WT-1 / 280-

WT-1 / 441-

37.70

100.20

146.86

87.62

93.04

43.79

69.78

93.01

70.03

95.63

45.66

137.52

42.32

118.12

220.67

144.38

54.04

30.79

136.28

97.86

105.90

70.42

172.31

++

++

++

++

++

++

++

++

++

A2 Binding Titration

2

peptides

WT1

10

17

126

187

225

235

280

441

HPVE7 EG-93

HBV 17-28

BEST AVAILABLE COPY

Plate peptide in RPMI + 5% BSA/FCS in 96 well plate (U bottomed). from 100 μ M of first column to column 11 (0.0001 μ M) in 50 μ l medium. Add 50 μ l of T2 cells at a conc of 6×10^6 /ml. 5×10^5 /well mix well.

37°C overnight.

wash x 1 \pm PBS (cold) + 1% FCSStain \pm HB 117 and HB 54 (parent culture \pm) 5 min for 30' on ice.

wash x 3

Apply Ga m Ig FITC at 1/250 (50 μ l) for 30' on ice

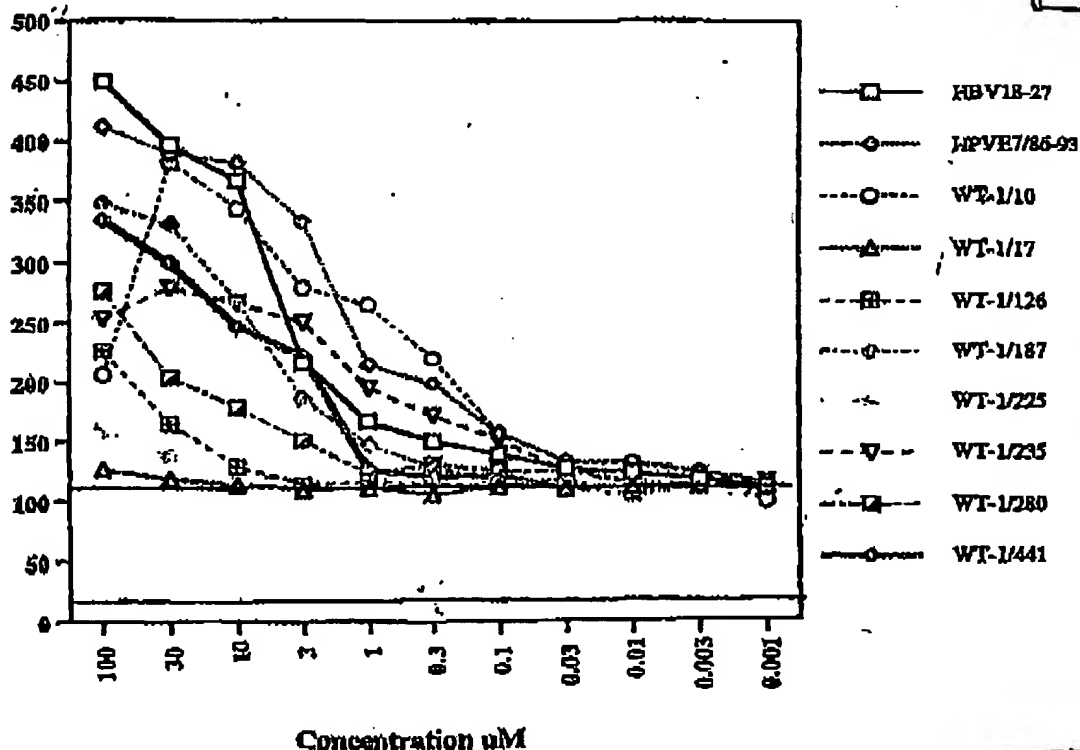
wash x 3

Analyse on FACS

Results: Good. See attached sheets.

BEST AVAILABLE COPY

WT-1 peptides binding to A2 molecules on T2 cells
 --- Stained with HB54 mAb



Binding of WT-1 peptides to A2 molecules on T2 cells
 --- Stained with HB117 mAb

